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BOTANICAL GAZETTE

MAY, 1903

ON THE RELATIONSHIP OF THE NUCLEAR MEMBRANE TO THE PROTOPLAST.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.
XLVII.

ANSTRUTHER A. LAWSON.

(WITH PLATE XV)

THE nucleus has long been regarded as not only the most important, but with its chromatin, linin, nucleolus, karyolymph, and membrane, it is also regarded as the most complex organ of the cell. Although the literature on plant cytology is rapidly accumulating and revealing much that is of interest on the centrosome question, spindle formation, chromosome reduction, etc., we as yet know little or nothing as to the origin and development of the nucleus. Its morphology is hidden by its complexity. This apparent complexity of the nucleus is in a large measure due to the presence of the nuclear membrane. A nucleus without a membrane, as we see it in certain stages of mitosis, becomes a comparatively simple structure. Such a condition of the nucleus may be observed immediately preceding spindle formation in the spore mother cells of any of the higher plants. At this stage the nuclear wall disappears, the karyolymph becomes diffused throughout the cytoplasm, the nucleolus and the linin lose their identity, and the only element left which can be regarded as nuclear is the mass of chromatin. It thus becomes evident that the formation of a membrane is the most important factor which gives the nucleus its complex structure.

So far as the writer is aware, no observations have been recorded on the origin and formation of the nuclear membrane in plants. It is the object of the present work, therefore, to account for the presence of this membrane and its morphological relationship to the other nuclear elements, with the hope that it might lead to at least a partial understanding of the morphology of the nucleus itself.

In undertaking this work it was at first thought that a study of the most primitive types of plant life—where the cell is much simpler and the nuclear structures less highly differentiated than in the higher types—would be the most natural way of approaching the subject. Consequently the cell contents of a large number of forms of the Cyanophyceae and of the simpler Chlorophyceae were examined. A careful study of these forms, however, failed to give satisfactory results so far as the formation of the nuclear membrane is concerned. In the cell of the Cyanophyceae bodies were found which resembled chromatin, but in none of the forms examined could any of the other nuclear structures be identified. These chromatin-like bodies were surrounded by neither nuclear sap nor membrane. In the simpler Chlorophyceae examined the chromatin was found to be surrounded by both nuclear sap and membrane, but the nucleus was found to be much too small to follow out accurately the stages in the formation of the nuclear membrane. It has been thought advisable, therefore, to use the highly organized nucleus of the flowering plants as an object for this study. The following conclusions, therefore, are drawn mainly from observations made in the individual history of the typical nucleus of the higher plants.

MATERIAL AND METHODS.

In order to follow the sequence of events which lead to the formation of the nuclear membrane, it is desirable to obtain material with clear large nuclei, where the various stages in the development of the daughter nuclei may be distinctly observed. For this purpose the sporogenous cells of the higher plants seemed to furnish all the desired conditions.

Although the spore mother cells of *Passiflora coerulea* and the

archesporial cells of *Equisetum limosum* were especially studied, and were selected as types to illustrate the process of nuclear membrane-formation, the results obtained from these forms were confirmed by observations made on several other forms, such as *Lilium*, *Cobaea*, *Gladiolus*, *Hedera*, *Pinus*, *Pteris*, etc.

Flemming's strong solution of chromic-osmic-acetic acid, diluted with one volume of water, was used for fixing, and the triple stain, safranin, gentian-violet, and orange G, for staining. After being fixed the material was washed in running water and was then dehydrated by being passed through various grades of alcohol. Bergamot oil preceded the infiltration of paraffin. Microtome sections from 1 to 3.6μ thick were used.

THE FORMATION OF VACUOLES AND THEIR RESEMBLANCE TO NUCLEI.

To any one who has made a special study of the nucleus, especially of the spore mother cells of the higher plants, the resemblance of this body to a vacuole has probably suggested itself. As is well known, the presence of a vacuole is one of the most striking characteristics of the plant cell. This is particularly so of the mature cells in any vegetative tissue, where the vacuole is quite as constant as the nucleus. The vacuole is not apparent in the very young cell, as the nucleus and cytoplasm seem to occupy the entire cell cavity. At an early stage in the growth of the cell, however, small lacunae containing a watery fluid may be observed in the cytoplasm. As the cell continues to grow these lacunae become much larger and finally flow together, forming one or more large vacuoles in the cell. As these vacuoles enlarge the cell continues its growth. The amount of cytoplasm, however, does not increase at the same rate as the contents of the vacuole. The result is that in the mature cell the vacuole may occupy the greater part of the cell cavity. The watery fluid of the vacuole holds many substances in solution. It may function as a storehouse for reserve food material. It may even contain solid bodies such as starch grains, aleurone grains, and crystals of calcium carbonate or oxalate. Finally the vacuole is always surrounded by a distinct limiting plasmatic membrane.

In all of these particulars the structure of the mature nucleus bears a very striking resemblance to the vacuole. It is a cavity containing a watery fluid—the nuclear sap or karyolymph—in which lie imbedded the chromatin thread and nucleoli. Structurally the nuclear sap may be compared to the cell sap of the vacuole; the chromatin and nucleoli may be compared to the solid bodies of reserve food materials found in the cell sap; and finally the nuclear membrane may be compared to the tonoplast. These two latter structures not only bear a general resemblance to each other, but, as we shall demonstrate later, their origin and method of formation are identical.

This resemblance of the nucleus to the vacuole is particularly evident in the cells of sporogenous tissue, where the vacuole is not usually present and where the nuclear cavity occupies the greater part of the cell. In many cases the nucleus was represented as constituting fully two-thirds of the cell contents (*fig. 13*), and the cytoplasm in such cases was a mere zone between the nuclear membrane and the cell wall. As we know so little in regard to the direct function of the nucleus in relation to metabolism, we are unable to account for the large size of the nuclear cavity in these sporogenous cells. But as these cells are characterized by the absence of a vacuole, this fact suggests that the nuclear cavity may here function as the vacuole functions in the vegetative cells. The writer regards this suggestion worthy of consideration, since the function of the nucleus is coming to be regarded more and more as an essential factor in constructive metabolism.

THE PRESENCE AND FORMATION OF PLASMATIC MEMBRANES IN THE CELL.

Of late years much attention has been given to the importance of plasmatic membranes and their relationship to osmosis. As a result of the investigations of Nägeli, Traube, and more particularly of Pfeffer, the cell is now regarded as an osmotic system composed of a series of membranes. In the typical plant cell there are three of these membranes commonly recognized, namely, the cell wall, the ectoplast, and the tonoplast.

Structurally the cell wall is entirely different from the ectoplast and tonoplast. Composed mainly of cellulose, it protects the inner protoplast, gives rigidity to the whole cell, and allows of a considerable internal osmotic pressure. As its method of formation has nothing in common with that of the inner plasmatic membranes, its presence will not form a part of the present discussion.

The two inner plasmatic membranes—which in the ordinary vegetative cell constitute the outer and inner limiting layers respectively of the primordial utricle—have the same general structure, and their methods of formation are identical, so far as we know. The ectoplast is present in every cell, no matter whether the cell is surrounded by a cell wall or not. It is very conspicuous in naked cells. In the ordinary cell, when not in a state of plasmolysis, the ectoplast is always found in close contact with the cell wall. As far as can be revealed by the microscope, it is merely a differentiated film of the cytoplasm.

The tonoplast is always to be found surrounding the watery fluid of the vacuole. As young cells and most sporogenous cells do not possess a vacuole, the tonoplast is not present. It is not as constant a cell structure, therefore, as the ectoplast. It is, like the ectoplast, merely a differentiated portion of the cytoplasm. Its formation may be followed readily by observing the gradual development of the vacuole which is associated with the growth of all vegetative cells. A complete series showing the gradual formation of the vacuole may be observed in any growing region, such as root tips or growing points. Accompanying the gradual accumulation of the watery contents of the vacuole, the gradual differentiation of the cytoplasm coming in contact with the cell sap becomes quite evident. Just what changes in the minute structure of the cytoplasm take place in formation of the membrane cannot be revealed by the microscope. In this connection, however, Pfeffer says as follows:

Every mass of watery fluid present in the protoplasm must be surrounded by a vacuolar membrane to form a larger or smaller vacuole, while masses of plasma which have escaped from the cell also become clothed by a plasmatic membrane, and form large vacuolar bubbles in water, but not in plasmolysing solutions. From what has been said above, it follows that a plas-

matic membrane must be immediately formed on a freshly exposed surface as it comes into contact, not with plasma, but with other media and especially water. Nevertheless, the determining causes cannot at present be precisely defined, and it is hardly probable that the plasmatic membrane is simply the expression of the physical surface tension, which is necessarily always present. The latter may be of decisive importance, however, in the formation by means of the molecular forces exerted by it, while at the same time contact with the medium may cause the substances in the peripheral film to be precipitated in an insoluble form, which redissolves when returned to the interior of the plasma. Since a membrane is formed on every isolated fragment of plasma, even when its vital activity is reduced to its lowest ebb, it seems as if the actual exposure of a peripheral film induces the formation of the plasmatic membrane.

It is not my purpose to enter into a theoretical discussion as to the minute changes which take place during the formation of the plasmatic membranes, but merely to point out the fact that whenever the cytoplasm is exposed to a watery surface a membrane is formed and that all membranes thus formed are structurally identical, so far as can be revealed by the microscope. A third internal plasmatic membrane which has never been considered in this connection is the nuclear membrane. It too comes in contact with a watery fluid, the nuclear sap or karyolymph. As we shall see from the following observations, it is formed in identically the same manner as the tonoplast. It is nothing more than a modified film of cytoplasm, and on account of its similarity in structure and method of formation to that of the ectoplast and tonoplast the writer regards it as one of the series of plasmatic membranes of the cell.

THE FORMATION OF THE NUCLEAR MEMBRANE.

In the history of every nucleus of the higher plants which divides mitotically there are two stages in which this organ consists of nothing but chromatin. The first of these stages may be seen in any mother nucleus in its preparation for mitosis during the period of spindle formation. At this time the nuclear wall breaks down, the nucleoli and linin disappear, the karyolymph becomes diffused throughout the cytoplasm, and by the time the spindle is formed the only nuclear element that remains is the chromatin which has assumed the form of definite

chromosomes. In his works on *Cobaea* and *Gladiolus* (1898, 1900) the writer called attention to the breaking down of the nuclear membrane. Beyond stating that the observations made on these forms have been confirmed by a study of the material used in the present investigation, it will be unnecessary to describe the process farther. It might be well, however, to call attention to the fact that it seems to be a general occurrence in the higher plants that the nuclear membrane breaks down either immediately preceding or during the process of spindle formation, and that therefore the mother nucleus is at this period devoid of a membrane.

The second stage in which the nucleus is devoid of a membrane may be observed in the young daughter nuclei previous to the accumulation of the karyolymph. It is this second stage which affords us an opportunity of following the course of events which leads to the formation of a membrane around each daughter nucleus. *Fig. 1* illustrates a stage in the pollen mother cell of *Passiflora coerulea* immediately after the chromosomes have reached the poles of the spindle. Here the chromosomes are shown still attached to the connective fibers of the spindle, but otherwise lie freely in the cytoplasm. Their individual identity may be easily observed. This stage is of very short duration, however, for the chromosomes soon fuse together, forming a single mass of chromatin. This fusion of the chromosomes is shown in *fig. 2*. This large irregularly shaped mass of chromatin comes in contact with the cytoplasm at every point of its outer surface. With the hope of detecting the very first indication of the formation of the nuclear membrane, many preparations showing this stage were carefully studied, but in no case could any trace of a membrane be found during this period. The nucleus at this time consists of nothing but a large single irregularly shaped mass of chromatin.

This stage, however, like the first, is of very short duration. The chromatin undergoes a change which is accompanied by the appearance of small but very distinct lacunae within the chromatin mass. *Fig. 3* shows two of these lacunae centrally situated. This is the first indication of the accumulation of karyo-

lymph. These lacunae increase in size, flow together, and appear to force the surrounding chromatin outward. This gradual increase in the amount of karyolymph is well shown in *figs. 4* and *5*. *Fig. 4* shows a large central cavity in the chromatin mass, and the whole takes on a ring-like appearance in section. *Fig. 5* shows this still further developed, with two cavities filled with karyolymph. The chromatin here is doubtless preparing to take on the spirem condition of the ordinary resting nucleus. These stages (see *figs. 3, 4, 5, 6*) clearly show that this change in the shape of the chromatin is closely associated with the accumulation of the karyolymph. Up to the stage shown in *fig. 5* no change whatever could be detected in the cytoplasm that would indicate the formation of a membrane, although up to this time the chromatin has been in close touch, on all sides, with the cytoplasm. In *fig. 6*, however, is shown a stage in which the karyolymph has increased to such an extent and the chromatin has become so divided that the latter is no longer immediately surrounded by cytoplasm. Here for the first time the karyolymph comes in direct contact with the cytoplasm, and here also we have the first indication of a membrane. As shown in *fig. 6*, the membrane does not completely surround the chromatin, but is only observable at that region where the cytoplasm is directly exposed to the karyolymph. By the still greater increase in the amount of karyolymph which is accompanied by a still greater change in the shape of the chromatin (*figs. 7, 8*), this exposed region increases until the chromatin is completely surrounded by the karyolymph (*fig. 7*), and the latter becomes exposed to the cytoplasm on all sides. A complete series of stages was carefully examined, and it was found without exception that just as soon as a cytoplasm becomes exposed to the karyolymph there a membrane is formed, and where this exposed surface grows by the greater accumulation of the karyolymph the membrane increases accordingly. The result of this is a large central vacuolar structure (*figs. 8, 13*) containing chromatin in the spirem stage and commonly recognized as the resting condition of the mature nucleus.

While the formation of the nuclear membrane was most

carefully followed in the pollen mother cells of *Passiflora*, and is here taken as the type, many other forms were also studied. Among these were the pollen mother cells of *Cobaea*, *Gladiolus*, *Hedera*, *Lilium*, *Smilacina*, and the archesporial cells of *Equisetum*. In all of these forms the process of nuclear membrane-formation was found to be practically the same as that described for *Passiflora*. In certain cases, however, it was found that in the accumulation of the karyolymph and the dividing of the chromatin mass the process differs slightly. This was particularly evident in the archesporial cells of *Equisetum limosum*. Here, as in *Passiflora*, the chromosomes unite to form a single mass of chromatin at each pole of the spindle (*fig. 9*). In the next stage, however, instead of only two or three lacunae appearing in the chromatin, a large number make their appearance (*fig. 10*). These increase in number, grow independently, and do not flow together in this early stage as they do in *Passiflora*. The result is—as shown in *figs. 10, 11, 12*—that in section view we do not have that ring-like appearance of the chromatin which was found to be so characteristic of *Passiflora*.

Fig. 11 shows a condition in which the lacunae have increased to such an extent as to extend beyond the chromatin, and the contained karyolymph is therefore directly exposed to the cytoplasm in these places. The result here, as shown in *fig. 11*, is an incomplete membrane surrounding each daughter nucleus, with the membrane only visible in those regions where the cytoplasm is exposed to the karyolymph.

From the above observations it would seem that as the chromatin of the daughter nucleus divides up in its preparation to pass into the spirem condition there is secreted a watery fluid commonly recognized as the karyolymph or nuclear sap. The karyolymph is first contained within the chromatin mass, but its volume increases and extends beyond the chromatin and consequently comes in contact with the cytoplasm. There seems little question that it is the exposure to the cytoplasm which causes the formation of a membrane around the nucleus, and the nuclear membrane is therefore a cytoplasmic structure.

GENERAL CONCLUSIONS.

It is quite natural to suppose that the secret of the origin of the cell nucleus can only be revealed by a study of the most primitive types of life. That such a supposition has influenced many investigators becomes quite evident when one glances over the enormous amount of literature that has accumulated on the cytology of the Bacteria and the Cyanophyceae. On these primitive forms a great deal of work has been done with the hope of throwing light on the origin of the nucleus. Through the efforts of Bütschli (1890, 1892, 1896), Fischer (1891, 1897), Zacharias (1887, 1890, 1897), Pella (1894), Macallum (1895, 1900), and many others, much valuable data has been accumulated, but as yet no satisfactory conclusions have been reached. This failure is probably due to the fact that many investigators have not a correct morphological conception of the nucleus as we find it in the higher types of life.

As a result of the above studies on the formation of the nuclear membrane, the writer's conception of a nucleus has been considerably modified. Instead of the nucleus being regarded as a sac containing karyolymph, chromatin, nucleoli, linin, and a membrane, the chromatin alone is regarded as the only permanent morphological constituent of the nucleus. The karyolymph and the membrane are merely temporary physiological results. The accumulation of the nuclear sap is the result of protoplasmic activity. It is first secreted within the chromatin mass and later surrounds it. The cytoplasm coming in contact with the nuclear sap forms a membrane in precisely the same manner as it forms the tonoplast when it comes in contact with the cell sap of the vacuole. The writer therefore regards the nuclear sap as no more permanent than the cell sap, and the nuclear membrane as no more permanent than the tonoplast. Furthermore, as we have shown that the nuclear membrane is of cytoplasmic origin, this structure is to be regarded more as the inner limiting layer of cytoplasm than as a constituent of the nucleus.

To convince one's self that the chromatin is the only permanent element of the nucleus, it is only necessary to observe the

various stages of mitosis. Immediately after the nucleus divides there are two masses of chromatin in the cell. For some time these masses of chromatin are surrounded by neither karyolymph or membrane, and yet no one will deny that they are nuclei. The law that the nucleus never arises *de novo*, but always from the division of a preexisting nucleus, does not hold good if we regard the membrane, karyolymph, or nucleoli as morphological elements. We must either change this law of the permanency of the nucleus or change our morphological conception of this organ. As there seems to be no doubt as to the permanency of the chromatin from cell to cell, and as the other constituents of the mature nucleus are physiological results which are entirely renewed with each succeeding mitosis, there is good reason to regard the chromatin alone as the nucleus, whether it is surrounded by karyolymph and membrane or lying freely in the cytoplasm.

The question as to the existence of a nucleus in the Cyanophyceae and Bacteria has been the subject of controversy for a great many years and even up to the present time it has not been satisfactorily shown that such an organ exists in the cells of these primitive organisms. Among the modern workers in this field, as Zukal (1892), Palla (1894), Nadson (1895), Bütschli (1892-1896), Zacharias (1897), Fischer (1897), and Macallum (1895-1900), a great divergence of opinion is expressed. Some claim that a nucleus is present; while on the other hand many claim that no structure exists that can be interpreted as a nucleus: others again have shown quite conclusively that chromatin-like granules are present, but hold that these do not represent the nucleus. Of the writers who hold this latter view Macallum (1900) expresses himself probably more strongly than the others. In his work "On the cytology of non-nucleated organisms" he finds that the cells of *Beggiatoa* and of the Cyanophyceae contain granules which stain like chromatin and give all its known reactions. Although he regards these structures analogous to chromatin, he concludes that "there is no nucleus nor any structure which resembles a nucleus in the Cyanophyceae." He holds a similar view in regard to the *Beggiatoa* cell

As the highly complex nature of the typical nucleus has doubtless been the result of the demand for a physiological division of labor in the cell, there is no reason why we should expect to find such a condition in the primitive cell of the Cyanophyceae or Bacteria. The majority of the writers who have investigated the cytology of these forms agree that chromatin-like granules exist in these primitive cells, but that these granules are surrounded by neither nuclear sap nor membrane, and many therefore do not regard these granules as representing the nucleus. As I have attempted to show that the so-called nuclear membrane is merely the inner limiting membrane of cytoplasm, and that every typical cell passes through a phase in its history when it consists of nothing but chromatin granules, the writer sees in this a possible reconciliation for the divergence of opinion that is held in regard to the nucleus of the Cyanophyceae. For might we not regard that stage of the daughter nucleus when it is devoid of a membrane as representing a phase in its ontogeny which approaches that primitive condition which we find in the cells of the bacteria and Cyanophyceae, although the chromatin-like bodies in the cells of these forms may not be the ancestral nucleus.

In conclusion, I take pleasure in acknowledging my indebtedness to Dr. Bradley M. Davis and Dr. Charles J. Chamberlin for advice, and to the Botanical Department of the University of Chicago for the excellent facilities for cytological research which were placed at my disposal.

SUMMARY.

The results of the above observations may be briefly summarized as follows:

The typical nucleus of the higher plants is a water cavity structurally similar to that of the cell vacuole.

The chromatin is the only permanent constituent of the nucleus. The karyolymph, linin, nucleoli, and membrane are renewed with each succeeding mitosis.

The nuclear membrane originates like the tonoplast. It is formed by the cytoplasm coming in contact with the karyo-

lymph just as the tonoplast is formed by the cytoplasm coming in contact with the cell sap.

The karyolymph is no more permanent than the cell sap, and the nuclear membrane is no more permanent than the tonoplast.

As the nuclear membrane is of cytoplasmic origin, it is regarded as the inner limiting membrane of cytoplasm rather than as a constituent of the nucleus.

Although the chromatin granules found in the cells of the Cyanophyceae and Bacteria are surrounded by neither karyolymph nor membrane, these granules nevertheless represent the nucleus, since every highly organized nucleus passes through a stage in its development when it consists of nothing but chromatin.

It is further suggested that the primitive nucleus probably did not secrete a karyolymph and therefore no nuclear membrane was formed.

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EXPLANATION OF PLATE XV.

The figures were drawn with the aid of Abbe's camera lucida, Zeiss homogeneous immersion, objective $\frac{1}{2}$, apert. 1.25, compensating ocular no. 8.

FIG. 1. A portion of a pollen mother-cell of *Passiflora coerulea* during nuclear division; the figure shows the chromosomes of one daughter nucleus at the poles; the connective fibers are still attached to the chromosomes but otherwise the latter lie freely in the cytoplasm.

FIG. 2. A later stage of the same; the chromosomes have fused together and the daughter nucleus is represented as a large single irregularly shaped mass of chromatin.

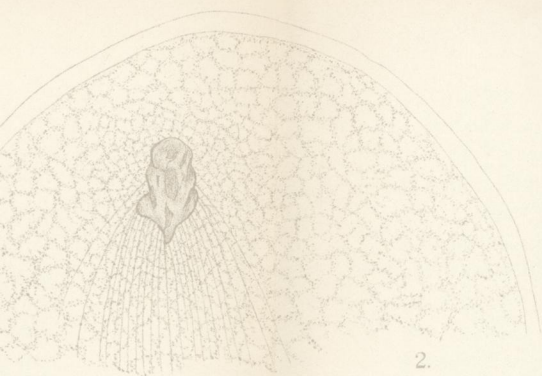
FIG. 3. Shows the presence of small lacunae within the chromatin mass of the daughter nucleus; these lacunae are the first indications of the accumulation of the nuclear sap or karyolymph.

FIG. 4. Shows an increase in the amount of karyolymph; the smaller lacunae have united, forming one central cavity and the chromatin has the appearance of a sphere with the karyolymph in the center; in section the daughter nucleus has the appearance of a ring of chromatin surrounding a central cavity of karyolymph.

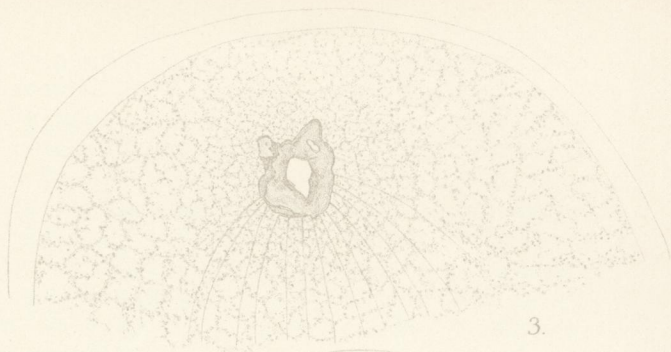
FIG. 5. Shows a somewhat later stage than *fig. 4*; the karyolymph has increased in quantity and the chromatin has undergone still further modifications in its shape; up to this stage no trace of a nuclear membrane could be detected; the chromatin lies freely in the cytoplasm.

FIG. 6. This shows a stage in the daughter nucleus where the karyolymph has increased to such an extent and the chromatin has become so divided that the latter is no longer completely surrounded by cytoplasm; as a result the cytoplasm is for the first time exposed to the karyolymph; at the region

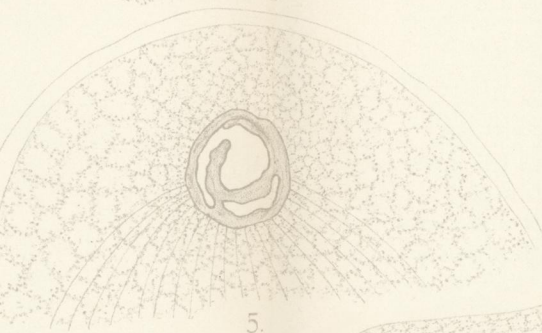




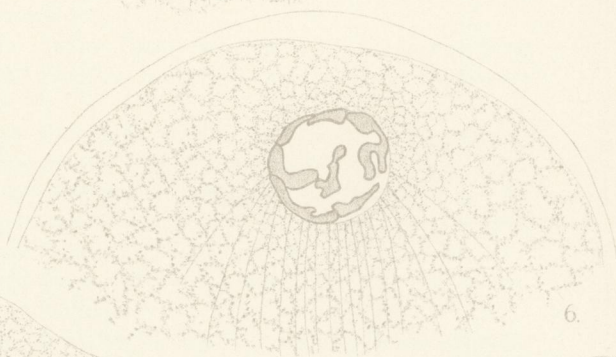
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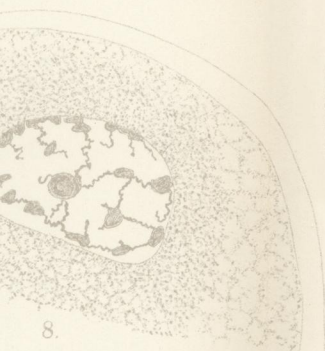
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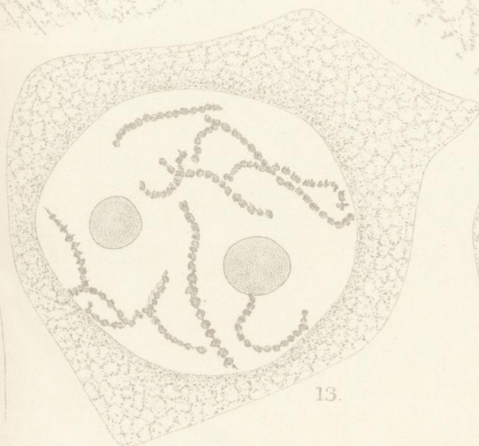
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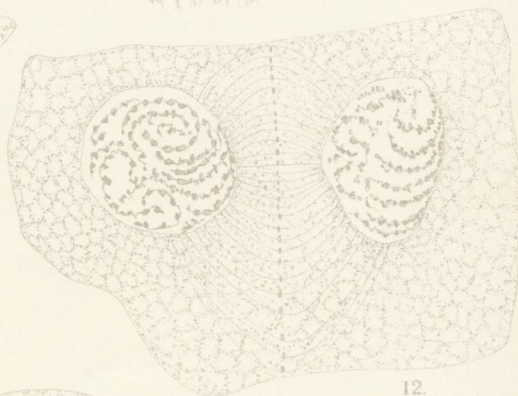
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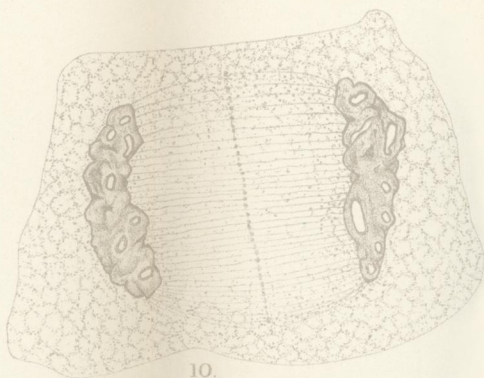
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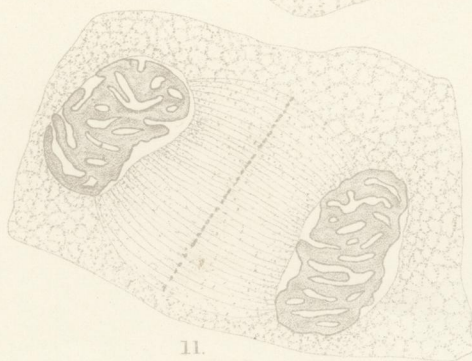
13.



12.



10.



11.

of contact of the karyolymph with the cytoplasm we can observe the first indication of the nuclear membrane.

FIG. 7. Shows a still later stage where the karyolymph has increased considerably and the chromatin has become divided to such an extent as to expose the cytoplasm to the karyolymph on all sides, and as a result we have a complete membrane formed; the daughter nucleus is now perfectly spherical, containing all the elements of a mature nucleus.

FIG. 8. Shows a daughter nucleus with the chromatin in the spirem stage; this is after the cell plate has been formed and the accumulation of the granular zone around the daughter nucleus indicates that it will soon prepare for the second division.

FIG. 9. An archesporial cell of *Equisetum limosum*; the chromosomes have fused together and each daughter nucleus consists of a large irregularly shaped mass of chromatin.

FIG. 10. The same at a later stage, showing several lacunae containing karyolymph within the chromatin mass of each daughter nucleus.

FIG. 11. A still later stage showing an increase in size and number of the lacunae; some of the latter have increased to such a size as to extend beyond the chromatin, and as a result the cytoplasm becomes exposed to the karyolymph; the result is the appearance of a membrane at the region of contact of the cytoplasm with the karyolymph.

FIG. 12. Shows a stage where the volume of karyolymph has increased to such an extent as to completely surround the chromatin; the cytoplasm comes in contact with the karyolymph on sides resulting in the formation of a complete membrane about each daughter nucleus.

FIG. 13. Showing the vacuolar appearance of the mature nucleus of the archesporial cell of *Equisetum*; it also shows the relative size of these nuclei to the cytoplasm.